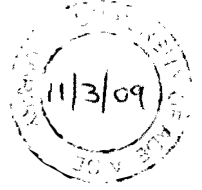


The development of targeted adenoviral vectors for gene therapy of vascular disease, with emphasis on the pulmonary vasculature.



A body of work submitted for the
Degree of Doctor of Medicine.

University of Adelaide, Department of Medicine.

by

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Copies of Publications

Introductory Chapters

1) *Reynolds PN*, Hemminki, A and Curiel DT. Gene Therapy. In Cecil’s Textbook of Medicine. 22nd Edition. Goldman L, and Ausiello D Eds. WB Saunders. 202-206. 2003.
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1) *Reynolds PN*, Miller CR, Goldman CK, Doukas J, Sosnowski BA, Rogers BE, Gomez-Navarro J, Pierce GF, Curiel, DT and Douglas JT. Targeting adenoviral infection with basic fibroblast growth factor enhances gene delivery to vascular endothelial and smooth muscle cells. Tumor Targeting 3: 156-168. 1998.
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7) Work LM, Ritchie N, Nicklin SA, *Reynolds PN, Baker AH. Dual targeting of gene delivery by genetic modification of adenovirus serotype 5 fibers and cell-selective transcriptional control. Gene Therapy, Aug;11(16):1296-300, 2004.*

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9) Miller WH, Brosnan MJ, Graham D, Nicol CG, Morecroft I, Channon KM, Danilov SM, *Reynolds PN, Baker AH, Dominiczak AF. Targeting endothelial cells with adenovirus expressing nitric oxide synthase prevents elevation of blood pressure in stroke-prone spontaneously hypertensive rats. Mol Ther. Aug;12(2):321-7, 2005.*

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Abstract / Summary

The development of gene therapy for clinical use continues to face many hurdles. A major issue is the limitation of gene delivery technology. This body of work describes strategies for improving the selectivity and efficacy of gene delivery to vascular endothelium, with emphasis on delivery to pulmonary vasculature in vivo. Several important principles were established which continue to be of relevance to the field. The work progresses from vector development through to the use of new vector strategies in the application of novel gene delivery approaches in disease models. Work in gene therapy and vector development began in the Division of Human Gene Therapy, University of Alabama at Birmingham, under the mentorship of Prof David T Curiel and has continued through international collaborations and the establishment of my own laboratory in the Hanson Institute with affiliate links to the University of Adelaide.

The work presented in this thesis consists entirely of published material, either as book chapters (three) or peer reviewed journal articles (twelve). The sequence of material progresses from a broad introduction to the field on Gene Therapy, more specific chapters dealing with pulmonary gene delivery including a detailed methodology chapter. The peer reviewed works contain an evolution of work dealing with the development of strategies to target adenoviral gene delivery vectors to the pulmonary vascular endothelium. This work encompasses the use of bi-specific conjugates, genetic modification of viral capsid (outer coat) proteins and the use of cell-specific promoters. The work progresses to a demonstration of the therapeutic gains achieved with the use of targeted over non-targeted vectors in animal models and culminates with a highly novel application of modulation of the bone morphogenetic protein pathway in pulmonary hypertension. A component of the work focuses on enhanced gene delivery to vein grafts ex-vivo.

There are many key original contributions encompassed within the work, including 1) first use of conjugate-based retargeting to vascular cells, 2) first demonstration that tropism modification could alter in vivo biodistribution of virus, 3) first demonstration of cell-specific retargeting of adenoviral vector after systemic vascular injection in vivo (a technique still unsurpassed in the field), 4) first demonstration of the in vivo selectivity gains achieved by combined cell-specific promoters with viral retargeting, 5) first demonstration of therapeutic gains achieved by targeting in a vascular context and 6) first demonstration that modulation of the BMPR2 pathway can have a therapeutic impact in pulmonary hypertension. Importantly, the targeting work I have developed has been adapted and used by others and laid a foundation for further vector improvements.